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ABSTRACT

Sausage is ready to eat meals for children and adults. The meat contained in a beef sausage might not be consistent to the one listed on the label. FTIR (Fourier Transform Infrared) spectroscopy combined with a chemometric method was the most used methods to detect beef fat with rapid and consistent results. Results of analysis can classify fatty acid composition. The aim of this study was to know the profile of spectra and grouping of beef tallow and dog meat with FTIR. The study was designed by making 7 different variations of dog reference sample concentrations (100%, 75%, 65%, 50%, 35%, 25%, and 100%) beef fat and five other samples were gathered from various street vendors. Results were analyzed using FTIR spectroscopy combined with chemometric with PLS (Partial Least Square) and PCA (Principal Component Analysis). The results of analysis will be analysed using Horizon MB application to obtained optimal wave number at 688-1124 cm^{-1}. Results equation obtained y = 0.9999 x + 0.0004 and value R^2 equal to 0.9999; RMSEC (*root mean square error of calibration*) of 0.30%; RMSEP (*root mean square error of prediction*) of 0.05% and RMSECV (*root mean square error of cross validation*) of 0.05%. The low values of RMSEP, RMSEC, RMSECV and R^2 close to one indicate that the FTIR combined with chemometric is an appropriate method for analyzing the presence of fat content in the sample. From the analysis showed that 1 of 5 sausage market samples has similarities with dog fat.

Keywords: beef fat, dog fat, sausage, FTIR, chemometric

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INTRODUCTION

Food is a basic necessity for human beings, so the availability of food need serious attention in quality & quantity. Foodstuffs can be derived from crops or livestock. Livestock products are the main source of nutrition for growth and human life (Gustiani, 2009). One of the livestock products containing high nutritional value are meat. With increasing people's income caused the demand for consumption of meat in some areas of Indonesia are increasing (Syahariza *et al*., 2005). Increased consumption of meat in Indonesia not only include halal meat but also non halal meat, like in some regions local food (Rafi *et al*., 2016). It can be seen from the large number of processed dog meat sellers and dogs provided each week for slain.

The current counterfeiting halal meat using a dog meat quite profitable since the trade of wild dogs in several countries is done at low prices. "Pikiran Rakyat" in December 2016 mentions the discovery of counterfeit products of beef using a dog meat in Bandung Raya (Sukirman, 2016). In addition to food safety, the halal food products are a factors that should also be a concern. Current awareness of muslim societies to consume halal food increased along with public awareness of Islamic laws (Che Man *et al*., 2005; Mursyidi, 2013; Rohman *et al*., 2014). Thus, it is necessary to know the laboratory testing posed the possibility of forgery meat precisely and accurately.

Laboratory testing for the presence of counterfeit used FTIR combined with chemometric (Jaswir *et al*., 2003; Rohman *et al*., 2012; Guntarti *et al*., 2015). The advantages of this method are simple, non destructive, sensitive, little sample needed, and does not involve the preparation of complex samples (Ronggo *et al*., 2007; Rahul *et al*., 2012). Data processing combined with chemometric to obtain important information about a particular object on the data using mathematical statistical techniques. Types of chemometrics that most commonly used are (1) grouping techniques, such as Principal Component Analysis (PCA) and (2) quantitative analysis with multivariate calibration, such as Partial Least Square (PLS) (Miller and Miller, 2005).

This research aims to know the profile of spectra and grouping of beef tallow and dog meat with FTIR. In addition to knowing whether or not there are impurities in the processed beef sausage that is on the market. The results of this research can be used as preliminary research to find out the content of non-halal food products.

MATERIALS AND METHOD

Tools and materials

The materials used in this research was fat from beef and dog sausage, *n*-hexane, anhydrous Na_2SO_4 and sausage sample market (Munawaroh and Prima, 2010). FTIR spectrophotometer instrument 3000 MB ABB (Canada) equipped with ZnSe Crystal plate, deuterated detector triglycine sulfate (DTGS) sample handling techniques with attenuated total reflectance (ATR), as well as x-ray beam splitter in Germanium KBr substrate. Droplet of oil placed on the ATR crystal controlled temperature (20°C). The measurement was done at 32 scan, separating power (resolution) 4 cm^{-1}, glass tools, Soxhlet (Guntarti *et al*., 2015).

Research Prosedure

Meat Supply

Dog meat was obtained from Kasongan, Bantul, Yogyakarta and beef meat from market. Aside from that, sausage products were also obtained from street vendors and traditional markets in Yogyakarta.

Sausage production

Grounded beef was measured according to the formula, and then it was mashed with a blender. After that, excipient was added, including flour, garlic, and eggs.

Fat extraction

Beef and dog meat were measured according to the formulation, and then chopped and mashed, then strained and put in Soxhlet. Solvent n-hexane was added twice. Extraction using Soxhlet was done for 5-7 hours and the temperature was maintained in 70ºC. The finished results were fat containing n-hexane solution. Then, Na_2SO_4 anhydrate was added and filtered using strain paper. Obtained fat was moved into effendorf to be further analyzed with FTIR (Guntarti *et al.*, 2015).

Data Analysis

Results of FTIR spectrum then analyzed using chemometric program with HorizonMB software, Microsoft Word and Microsoft Excel 2007. The samples were analyzed at 4000 cm^{-1} to 650 cm^{-1} wavenumbers. Multivariate analysis including calibration with PLS and PCA performed with HorizonMB program.

RESULTS AND DISCUSSION

Extraction of Fat

Extraction of fat by Soxhlet based on solid-liquid extraction. It used n-hexane solvent because its more stable, rigid, has a non-polar solubility, low boiling point, and low price (Munawaroh, 2010). It difference in organoleptic between dog and beef fat in colour and odour shown are sausage (Table I).

Table I. Identification of the type of beef and dog fat in sausages samples

Type Fat	Consistency at Room temperature	Colour Fat	Fatty Odour
Beef	Solid	Yellow	Not odorless
Dogs	Liquid	White	Typical smell

Based on the Table I, the differences can be seen between beef and dog fat in sausage. The beef fat in sausage has the consistency of a solid at room temperature, while the dog fat in sausage has a liquid consistency at room temperature. The difference in consistency on both types of fat content due to the constituent fatty acids on dog fat has many double bonds. The more number of double bonds in fatty acids, then the lower its melting point (Kusnandar, 2010).

Identification of the functional groups of beef and dog fat in sausages

Infrared spectrum analysis is unique, thus it's usually called fingerprint analysis. Different kind of substance will create different intensity, peak value, or wave number of extract for every peak. The reading of IR spectrum was done by mid-wave number, which was 4000-650 cm^{-1}. Mid-wave number was chosen because it can give sufficient information of the functional groups within the lipid. The difference between Beef Fat Spectrum (BS) and Dog Fat Spectrum (DS) at 100% are shown in Figure 1.

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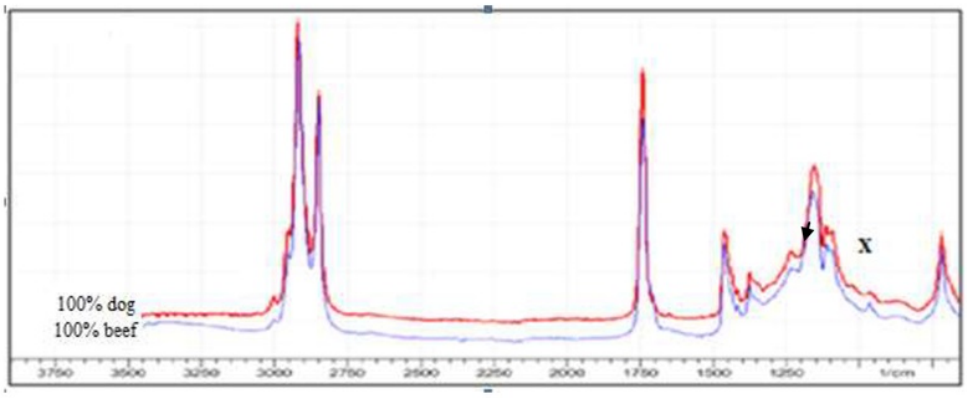

Figure 1. The difference between Beef Fat Spectrum (BS) and Dog Fat Spectrum (DS) at 100%

At a glance there's no significant difference between the spectra of fatty BS and spectra of DS. There was stretching CH double bond in the 3006 cm^{-1}. The existence of vibration of the Cis – C = CH on the numbers on the spectra and DS identifies the presence of unsaturated fatty acids on fat. On the wave number 1743 cm^{-1} there was a peak which indicates the carbonyl group (C=O) on the BS and the DS. Both of these fats may occur because the basic structure of fat triacylglycerols that contains 3 pieces of the carbonyl group of the esther. The peaks at wave number 1097 cm^{-1} slower output of the C-O from the cluster of esther.

The typical fat in beef and dog was in wave number 1031 cm^{-1}, peak (x) was only found in the dog fat whereas in beef fat not contained. On the wave number 1157 cm^{-1} there was vibration buckling of – CH in those wave numbers on plane. On the wave number 2921cm^{-1} on both fat there are vibrations of asymmetric and symmetric methylene ($-CH_3$). There was no significant difference between spectra BS and DS. The analysis of functional group of Beef Fat Spectrum and Dog Fat Spectrum as well as IR vibration are shown in Table II.

Table II. The analysis of functional group of beef fat spectrum and dog fat spectrum as well as IR vibration

Wave number (cm^{-1})	Functional Group Vibration	Intensity
3004 and 3006	*Cis* C=CH stretching	Peak
2955	Asymetric stretching vibration of methylen group ($-CH_2$)	Medium
2919 and 2920	Asymetric and symetric stretching vibration of methylen group ($-CH_2$)	Strong
1742 and 1743	Functional carbonyl group (C=O) of triacylglycerol esther bond	Strong
1377	Symetric bending vibration of methylen group ($-CH_2$)	Medium
1235	Stretching vibration of C-O group in esther	Medium
1158 and 1161	Bendring vibration of –CH in plane group	Strong
1097 and 1098	Stretching vibration of C-O from esther bond	Medium
964 and 965	Bending vibration of CH functional group of isolated trans-olefin	Medium
720 and 721	Overlapping vibration of methylen ($-CH_2$) and out of plane vibration by cis-distribution	Medium

Quantitative Analysis using PLS

Partial Least Square (PLS) was used as a quantitative analysis of beef fat and dog fat in the preparation of sausages. FTIR spectrum of Beef Fat Spectrum and Dog Fat Spectrum in gradual concentration in the middle infrared area (4000-650cm^{-1}) are shown in Figure 2. Results showed in wavenumber optimization in area 1124-688 cm^{-1} . This wavenumber has an unique functional group of dogs meat 1124-688 cm^{-1}. Aside the wave numbers, there is a significant difference between the BS and DS. This is in accordance with Ratnasari (2016), which states that the number of areas that demonstrate the specificity of dog fat in the wave number 1123 cm^{-1}. The results of optimization showed that wave number 1124 - 688 cm^{-1} generated value R^2 = 0,9999 and RMSEC = 0,30%. The results amounted to 0.05% RMSEP and RMSECV value of 0.05%. The value of RMSECV and RMSEP were validation parameters.

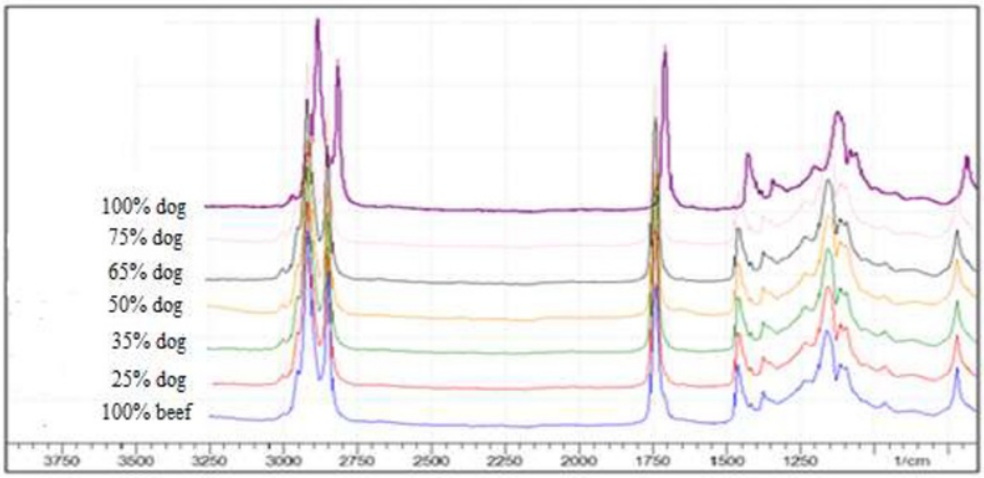

Figure 2. FTIR spectrum of beef fat spectrum and dog fat spectrum in gradual concentration in the middle infrared area (4000-650cm^{-1})

Classification of Fatty Acid using PCA

Principal Component Analysis (PCA) was a data interpretation method that done by data prediction. The amount of variable in a matrix was reduced to generate a new variable by maintaining the information of the data. The new variable was in the form of scores or main component. This technique can decrease the effect of noise and utilize the slight differences of IR spectrum. To analyze PCA, Beef Fat Spectrum 100% samples and Dog Fat Spectrum 100% samples from sausage extraction were used. PCA chemometric can be used to classified beef and dog lipid (Miller and Miller, 2005). In this research, it can be known that both samples was located on a different quadrant. Where the distance between the plot shows the similarities between the fat. It can be seen that the distance between the dog and beef fat separate long (Figure 3).

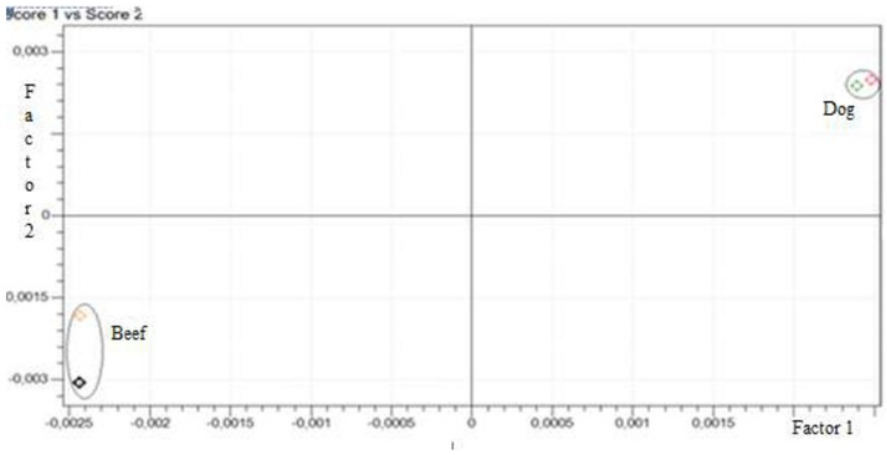

Figure 3. Graphic of score plot of beef and dog fat in sausages

Analysis of sausage products circulating in the market

Sausage samples in the general market was obtained from traditional market vendors. Spectrum was measured in an optimized wavenumber, which was 1124-688 cm^{-1}. The results of PCA analysis in a Score Plot are shown in Figure 4.

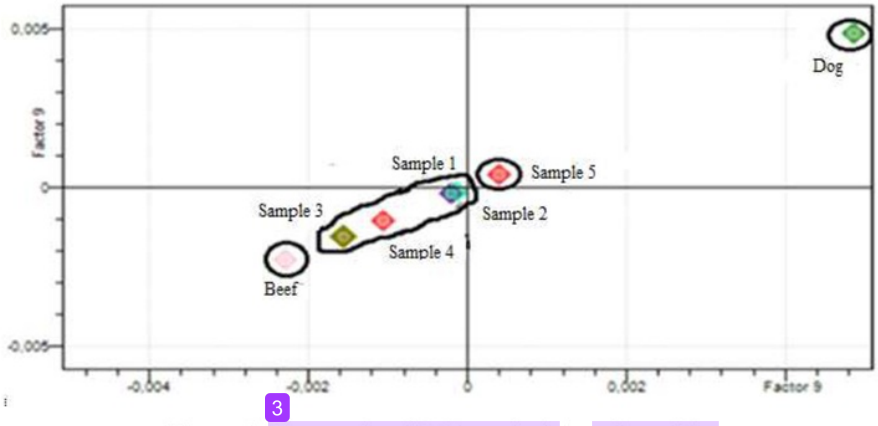

Figure 4. The results of PCA analysis in a Score Plot

In Figure 4, sample 1, 2, 3 and 4 adjacent to the beef fat. The closer the distance of score sample plots then the greater the likelihood that the sample contains pure beef. Where as on sample 5 adjacent to the beef fat but are on a regional score of dogs, so the plot it was possible that the suspected market sausage contains a mixture of beef and dog meats. So further research is needed to prove the truth of the existence of a mixture of beef and dog meats on the market samples.

CONCLUSION

Spectophotometry FTIR combined with chemometric Partial Least Square (PLS) was found in wave number area 1124-688 cm^{-1}. Calibration model with value R^2 0.9999; and RMSEC 0.30%. The results of validation model were obtained from RMSEP value 0.5%; and RMSECV value 0.05%. PCA multivariate analysis could classified beef and dog fat sausages. The analysis results of sausage samples shows that in the market there is one sample that contain dog meat.

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